SECTIONING PARAFFINE AT A TEMPERATURE OF 25° FAHRENHEIT.

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It is with some hesitation that we publish our troublesome device for cutting thin sections of the egg of *Allolobophora*, for in other material investigators are able to obtain thin sections without recourse to such complicated methods.

Our method is quite opposed to established rules, for the best authorities on technique recommend sectioning in a temperature varying, according to the season, from 60° to 70° Fahrenheit, in some cases employing heat reflected from a lamp, and they advise the use of paraffine having a melting point not higher than 45° to 50° C., emphasizing the fact that harder paraffine is "most hurtful to tissue." [1]

We found it necessary, however, to use paraffine of a much higher melting point, working slowly toward the use of this harder paraffine, and comparing the results step by step, in order to demonstrate whether the hard paraffine was really harmful to the egg structures. As no injury to the cytoplasmic or nuclear structures could be detected, we finally imbedded the eggs in the hardest paraffine obtainable, that having the melting point registered at 74° C. With this paraffine, a Thoma microtome, and the knife in the best possible condition, we were able to get moderately good sections of 5 and 6 μ, but an attempt to cut thinner sections crushed the paraffine enough to destroy the spherical form of the egg. What was gained by the thinner sections being more than sacrificed by the distortion of nearly all the constituents of the egg.

We tried to increase the hardness of the paraffine by devising an object carrier that would hold a piece of ice or iced water, but there was little gained by this method. The paraffine could be safely cooled only to a very limited degree below the temperature of the room, beyond this, moisture formed on the block and

[1] Bolles Lee, "The Microtomist's Vade-Mecum." 1896.

serial sections were impossible. We also tried Bütschli's method of painting each section with a thin layer of colloiden. In this way Bütschli obtained satisfactory sections less than 1 μ thick, but we failed to get like results.

We were convinced that the difficulties were not due to mechanical defects in the microtomes, for we were able to cut sections 3, 2 and even 1 μ with almost every microtome we have tried. But in these thin sections, the perfect contour of the eggs was destroyed, their diameter in some cases being reduced more than one half. This we demonstrated by cutting a series of sections, the first half dozen 10 μ, the next half dozen 5 μ, then 4, 3, 2 and 1 μ. Comparing the last sections of the series with the 10 μ section showed how much the structure of the egg was sacrificed to the thin sections, and convinced us of the necessity of devising some special method to harden the paraffine in order to secure thin sections in every way up to the standard of the 10 μ section.

Experimenting with a block of pure paraffine less than one eighth of an inch square (without any imbedded object) and setting the microtome at 3 μ, we gradually lowered the surrounding temperature until each section of the paraffine maintained the exact size of the original block.

The text-figure on page 283 illustrates the freezer we finally designed to enable us to cut serial sections of 1½ to 2½ μ at a temperature about 25° Fahrenheit. The work table, on which the freezer is operated, is placed close to a north window, and on the table we put a heavy cotton pad, covering this with a heavy rubber sheet. Then the microtome (Thoma in our case) is set in place on the rubber sheet. Near the microtome we arrange the wooden object carriers, each with its subject ready for final cutting; the necessary number of clean slides and boxes (with close covers) just large enough to hold a slide.[1] Then the freezer is put in place over the microtome, the work table forming the bottom of the operating compartment *D*. The ice chamber *B* is then packed with alternate layers of cracked ice and salt, the corks tied securely in the hand holes *H*, and a rubber tube fitted over the drain tube *M* with the free end in a pail under the

[1] We use for this purpose the shallow tin boxes formerly made for typewriter ribbons.

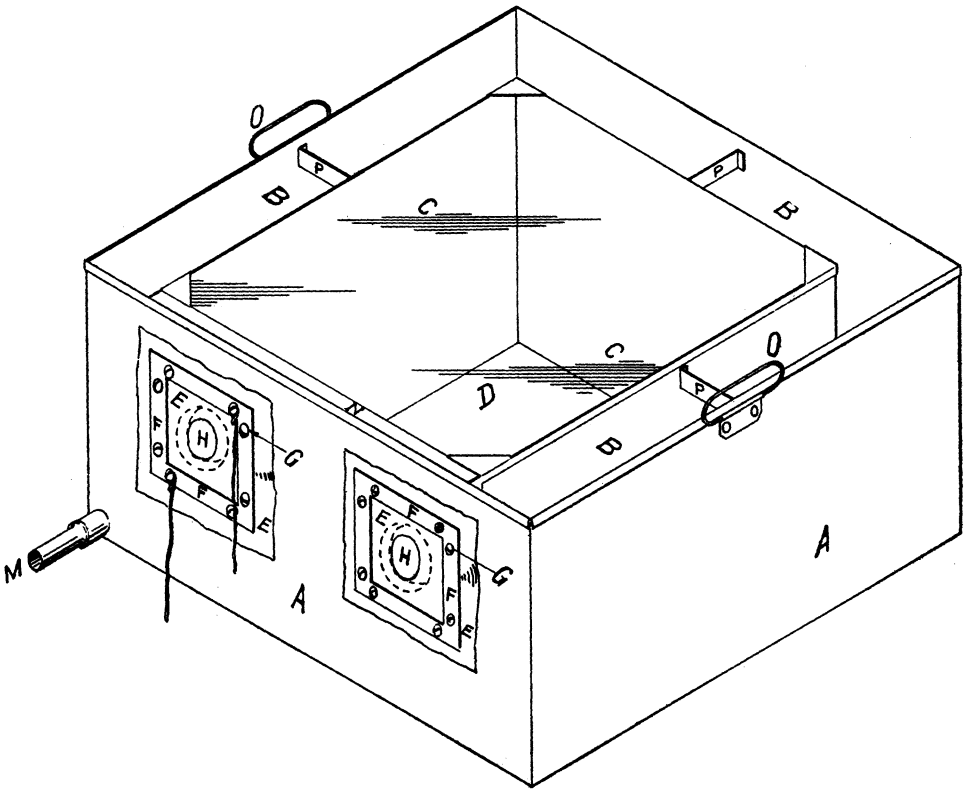

FIG. 1. The above figure is drawn to scale one eighth full size. *A*, exterior of copper box. *B*, ice chamber. *C*, plate glass ¾ inch thick, forming the top of operating compartment *D*. *D*, operating compartment The work table on which the microtome is placed forms the bottom of the operating compartment, which has the same area as the plate glass top, *C*. A thermometer is suspended in the compartment just above the microtome. This is done by glueing two loops of tape to the plate glass cover *C* and slipping the thermometer through these loops. We sometimes place a second thermometer on the bottom of the operating compartment, but the temperature registered by the one near the knife is the proper guide. *E*, heavy sheet rubber, which is kept in place by the copper frame, *F*, and copper bolts, *G*. *F*, copper frame. *G*, copper bolts which pass through the frame *F*, sheet rubber, *E*, and air chamber, *N*. The free ends of these bolts with nuts are in the operating compartment, *D*. *H*, hand holes in the heavy sheet rubber. The outer broken circle indicates the larger holes through the copper air chamber, *N*, and these must be large enough to admit the forearm freely. The hand hole, *H*, in the sheet rubber, must, on the contrary, be small enough to clasp the wrist tightly, to prevent escape of cold air from the operating compartment *D*. The sheet rubber must be renewed at least once a year, or as often as it becomes hard and loses its elasticity. When not operating the microtome, the hand holes, *H*, must be tightly closed with

table. After this the freezer must be covered with a heavy quilted covering and allowed to stand undisturbed for several hours. As a rule we "set" the freezer at night, and the following morning sectioning can begin at once.

When sectioning it is necessary to wear heavy gloves covering not only the hands, but the wrists and forearm. This obviates the discomfort of cold hands and prevents a too rapid rise of temperature in the freezer from the warmth radiating from the hands. To prevent frost forming on the glass cover, we have found it advisable to select cold days for sectioning, and to keep the window open and the temperature in the room below 50° Fahrenheit. The clear plate glass cover admits all the light needed for sectioning, but in setting the block a clearer view is obtained by reflecting the image from a small magnifying mirror.

As soon as a block is sectioned, the paraffine ribbon is lifted with a camel's hair brush to one of the cold slides in the freezer, the slide then carefully placed in one of the tin boxes and tightly covered. This is taken out of the freezer through the hand hole *H* and can be put in a cool place, fixing on the slide with the warm water method being deferred until all the blocks are sectioned.

After each block is cut, the next is immediately set for cutting, and the freezer again covered for ten or fifteen minutes. For during the process of sectioning the temperature in the operating compartment rises a few degrees, and the freezer must be kept covered until it has dropped again to 25° F. If while sectioning the knife becomes moist, the hands must be taken out at once, the hand-holes closed with the corks, and the freezer allowed to stand until the proper conditions of temperature are restored. Even the thinnest and longest ribbons do not snap or curl if the temperature in the operating compartment is not allowed to rise above the temperature of the knife.

In this way we cut from twenty-five to fifty blocks a day, all the material collected and imbedded during the summer requir-

large corks, held in place with two narrow tapes (shown in left opening). One of these tapes is passed through the ring of a screw in the center of the cork, and the tapes securely tied. If this is neglected the cold air in the freezer will force out the corks and cause a rapid rise of temperature in the operating compartment. *M*, drain tube fromi ce chamber. *N*, narrow air chamber, which is filled with cotton or some other non-conductor. *O*, copper handles. *P*, copper braces in ice chamber.

ing only a few days' work to prepare for study as needed. We always use very small blocks with four to six eggs arranged in a row in each block, and never more than one pair of ovaries in one block, as we find difficulties multiply with the increased size of the blocks, sections from the smaller blocks showing less tendency to curl or break.

It might seem that placing the microtome near an open window on a cold day would accomplish all we claim for the freezer, but the draught from an open window is sure to break the paraffine ribbon. Even in a cold room with closed windows, it is quite impossible to exclude currents of air, and the warm breath of the operator close to the microtome is a dangerous factor. In the freezer, the operating compartment is so nearly air tight that air currents are practically excluded. When the weather is intensely cold, we have sometimes used the freezer without ice, merely as a cover for the microtome, to enable us to section close to an open window, but we have always obtained the best results by using ice, and an even temperature as near 25° F. as possible.

For several years we have used this method of cutting thin sections of the egg of *Allolobophora*, for we had found it impossible to secure satisfactory thin sections of this egg with the methods in common use. Other investigators, however, rarely complain of difficulty in obtaining thin sections, and this may be due to fixing and hardening their material to a degree that makes any further hardening of the imbedded mass unnecessary, or to the fact that a large number of cells imbedded in one block of itself presents a harder mass than the single row of eggs we imbed in each block.

In our material these thin sections have been of the greatest value in aiding the interpretation of several obscure points; the constancy of the centrosome, for instance, was not demonstrated for this egg until we had secured sections of 3 μ or less. And for photographic reproduction at high magnification, thin sections offer very decided advantages, 3 μ representing the maximum thickness we have been able to use most successfully. It is possible of course to photograph the different planes of a thick[1] as

[1] With our method of focussing, a thick section requires a focussing (minus spherical) lens of lower power than the one used for thin sections.

well as a thin section, selecting the detail needed in each plane, but the structures above and below the selected plane being out of focus produce in thick sections a badly blurred image unfit for the best photographic reproduction.

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